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Kinetics of thermal degradation of beet dyes (*beta vulgares* l) in aqueous medium

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ABSTRACT
The betacyanin dyes present in beets (Beta vulgaris L) were extracted with water from lyophilized and pulverized beets, with a ratio of 50 g/1000 mL/10 minutes, at room temperature. The extract was separated by centrifugation at 3000 rpm/20 minutes. From the stock solution, dye solutions corresponding to the proportion of 5×10-3 gbeetroot mL-1 were prepared for the thermal degradation tests, in the temperature range of 30°C to 80°C, in a thermostatized bath, being monitored by absorption analysis in the ultraviolet-visible region, wavelength, □ , 480 nm for betaxanthins and 537 nm for betakinanins, for 180 minutes. The degradation of the dye follows a kinetic of 1st order for homogeneous reactions, with 1 reagent and the constant reaction rate was 1.4×10-4 mg/h, the half-life time, t1/2, was 8.25 hours, at a temperature of 30°C for the betacyanin dye. The degradation process has an activation energy of 55.39 kJ/mol for betacyanin dye and 56.06 kJmol-1 for betaxanthin. After the time of the degradation test, the solution assumes a yellowish color associated with betalamic acid, the degradation product of betacyanin.

Keywords: Beetroot, Betalaines, Thermal degradation.

1 INTRODUCTION

Industrialized products occupy an increasing share in the food market. They are very practical, as they already come ready or semi-ready, with numerous varieties of products for different types of consumers. However, along with this practicality, the use of chemical additives used to preserve or confer physical and chemical characteristics to foods, such as improvement in texture, color, flavor, increased shelf life, has its consumption put in check, regarding the daily consumption limit and its correlation with the appearance of adverse problems in the short and long term to the health of the consumer (LIMA, 2013; SOUZA, 2019).

Among the food additives, dyes are the most used by the industry (Resolution - CNNPA No. 44, of 1977). These are added to restore the tonality lost in processing, help protect smells and vitamins sensitive to clarity during storage and also serving as a visual indicator of quality, with color being a factor of fundamental importance in consumer choice (ANASTÁCIO, 2016; VALENTE 2018, Souza, 2019). Products with dyes look natural to the food, thus making it pleasing to the consumer's eyes (KONICA, 2021).

In general, many food products are naturally colored or added food colors that are made by combinations of substances, such as inorganic salts and volatile compounds. The dyes used in food can be natural dyes, dyes identical to natural, artificial and inorganic dyes (HAMERSKI, REZENDE & SILVA, 2013).

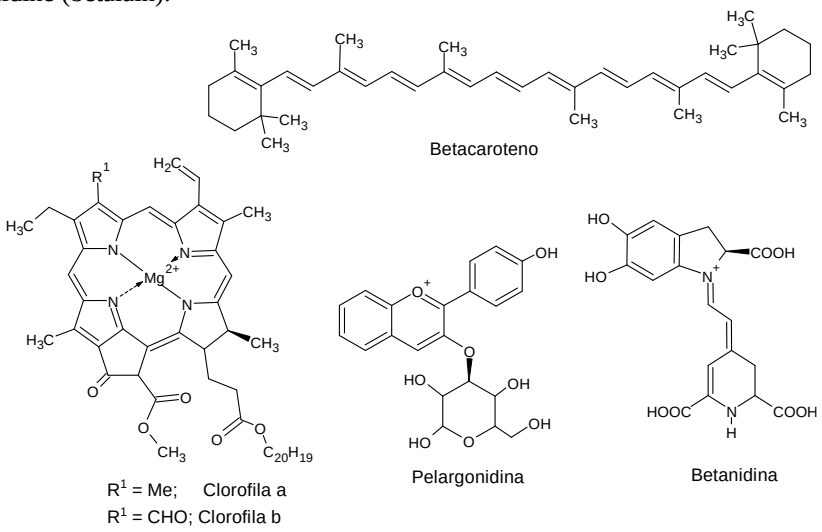
The use of synthetic dyes in excess can cause skin diseases and allergies, especially in children, causing gastrointestinal or respiratory symptoms. In addition, *in vitro* studies have already noted potential carcinogenic effects of these dyes (CORRADIN, 2019). With this, it has been perceived day by day the replacement of artificial colors by natural due to the rejection by consumers increasingly aware of the quality of what they consume (CAROCHO, 2015), who seek products with natural ingredients, free of artificial additives or any types of synthetic compounds (MARTINS, *et al*., 2019)

Natural dyes extracted from plant or animal origin, in addition to coloring, have beneficial properties for health, such as antioxidating, anticarcinogenic, antidiabetic, anti-inflammatory action and are also nontoxic and biodegradable (HAMERSKI, *et al*, 2013).

There is a wide variety of natural dyes with high potential for use applied to food.

Figure 1 shows some natural dyes found in plants.

Figure 1. Structure of plant pigments used in food. Chlorophyll (tetrapyrrole), pelargonidine (anthocyanin), beta-carotene (carotenoid) and betanidine (betalain).



Source: Gonçalves, 2015.

However, unfortunately they are unstable during processing and storage. Preventing undesirable changes is often difficult or impossible. Depending on the pigment, its use often requires stabilization procedures that allow maintaining its coloring capacity in different conditions such as the presence or absence of light, oxygen, temperature, water activity, pH and presence of other substances (AZEREDO, 2009; DAMODARAN, 2010).

Studies show that beets have multiple biologically active phytochemicals, including betalains, flavonoids, polyphenols, saponins, and inorganic nitrate, as well as several minerals. Its antioxidant power is attributed to betalains, which may attribute benefits against diseases related to oxidative stress. The betacyanin dyes (subdivided into betacyanin and betaxanthin) are bio-active compounds, being found in abundance in table beets (*Beta vulgaris* L*)* and are used as a natural colorant in the form of concentrated or powdered juice, in candies, ice creams, flavored milks and infant foods, among other foods (TIVELLI, 2011).

Red (or table) beets are an herbaceous plant in the family *Amaranthaceae*. It consists of a vegetable of purplish-red color, being three biotypes (sugar beet, forage and horticultural), of significant economic importance. Horticulture is the most used in Brazil. The bulbs (root) have thin bark, fibrous pulp and sweet and earthy flavor (TIVELLI *et al*., 2011, (IBGE, 2017a), CEAGESP (2021, (CAMPO & NEGÓCIOS, 2021)).

From a nutritional point of view, beets have high levels of antioxidants, vitamin B6, soluble fiber, folic acid, iron, zinc, calcium, phosphorus, sodium, niacin, biotin, potassium and magnesium (SANTOS, 2017). In Table 1 we can observe the centesimal composition of the root of the red beet (TACO, 2011, BOVI, 2019).

1.1 *BETACYANIN DYES*

Industrially, betalains are used as substitutes for artificial colors in the food industry. Its use is limited to foods with low moisture content and that do not have prolonged processing at high temperatures, such as ice cream, yogurts, flavored milks, meats and instant powders for desserts and puddings (LONGARAY, 2014; TIVELLI *ET AL*., 2011, GONCALVES, 2015; Henry, 1996).

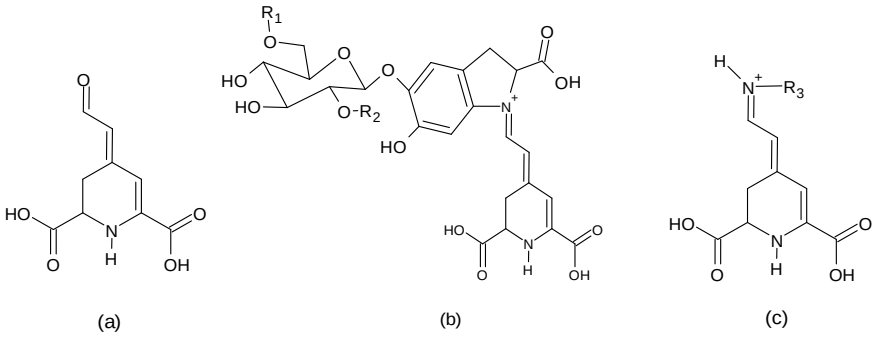
Beet dye is available for commercial use as a juice concentrated or powdered, in preparations containing between 0.3% and 1.0% of the betacyanin pigment considered the red extract of beetroot (DIAS, 2003). The dye in powder form has greater ease of handling and longer shelf life than alternatives such as the concentrate of this vegetable. Nowadays, the application of beet dye is quite varied, and can be used as additives in ice cream, yogurts, soft drinks, makeup among others.

Betalains, a class of natural pigments, comprise betacyanins (red) and betaxanthins (yellow). They occur mainly in *Centrosperm* with special emphasis on red beetroot (*Beta vulgaris* L.). Among betacyanins, the pigments that present the highest percentage (75-95 %) in red beets and stand out as a dye in foods are betanin and its isobetanin diastereoisomer. Betaxanthins appear in a smaller percentage in red beets, of which the main ones are vulgoxanthin I and II (JACKMAN and SMITH, 1992; MEGARD, 1993; STRACK, VOGT and SCHLIEMANN, 2003).

According to Delgado-Vargas (2000), more than seventy naturally occurring betalains are well known, and they all have the same basic structure (where R1 and R2 can be hydrogen or an aromatic substituent). However, betalains are divided into two groups with structural differences, betacyanins (with red/violet color and with a maximum absorption of 535-538 nm) that branch into four groups, betanin, gonfrerine, amaranthin and boungavilia; and betaxanthins (yellow in color).

The basic structures of betacyanins and betaxanthins, as well as their precursor, betalamic acid, can be seen in Figure 2 below.

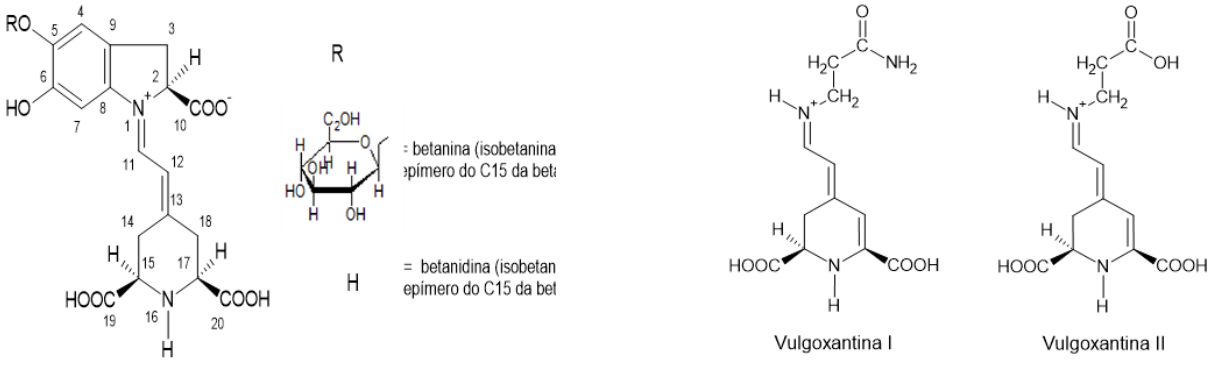
Figure 2. General structure of: (a) betamic acid, (b) betacyanins (c) betaxanthins (c). Betapenine: R1 = R2 = H. R3 = amine or amino acid group.



Source: Azeredo, 2009.

The structure of betanin and vulgoxanthin I and II, considered the most abundant dyes in red beets, are shown in Figure 3.

Figure 3. Chemical structures of the main betacyanins and betaxanthins.



Source: Drunkler, 2006.

Within the known betalains, there are about fifty structures of betacyanins and twenty of betaxanthins (VOLP, 2009). Alteration of the variable groups that constitute the pigment (R1 and R2) occurs due to the different sources from which these pigments can be obtained and, consequently, determine their tonality and stability (KHAN, 2016; SCHOEFS, 2004).

The susceptibility of betanin to heat, oxygen and high water activity restricts its use as a food coloring, being more used in products that have a restricted level of heat treatment, or that have low water activity, or even in foods with short shelf lives that do not include SO2 as a preservative in their formulation. In this way the most common products of application of this dye are the powder mixtures and frozen products (GONÇALVES, 2018; FRANCIS, 1989).

Thus, the research of new processes of extraction, conservation and modification of natural dyes represents an area of significant importance for the food industry, contributing to solutions of food processing problems and maintenance of the quality of industrialized products. Especially those aimed at children.

Within this context, this work aimed to evaluate the thermal stability of beet dyes (Beta vulgaris *L) in* aqueous medium by means of the kinetic study (n, k, t1/2, E*a) of the thermal degradation of betacyanin and betaxantinic dyes present in table beets in the temperature range of 30°C to 80°C.*

2 MATERIALS AND METHODS

2.1 PRE-TREATMENT OF THE RAW MATERIAL

The beets were acquired in the local trade in Imperatriz - MA, were washed, sanitized in sodium hypochlorite solution (0.05% w / v), for 15 minutes, peeled and grated with the aid of food processor (Skymsem, Model PA-7LE), using E1 cutting discs, with two passages of the material through the processor. It was then fractionated and packed in LDPE (low density polyethylene) bags, weighed, identified and stored in a freezer at - 21°C, until the moment of drying extraction.

The beet was freeze-dried (Liotope Lyophilizer, model L101) at -56°C/ 50 □Hg/50 h, obtaining the dry material. This was stored in glass jars, hermetically sealed, away from light, until the moment of extraction.

2.2 CHARACTERIZATION OF LYOPHILIZED BEET

The freeze-dried beet samples were submitted to moisture (IAL 012/IV method), acidity (IAL 016/IV method), protein (IAL 036/IV method) and crude fiber content (IAL 044/IV method) analyses.

2.3 EXTRACTION OF DYES

The freeze-dried beet was sprayed in a mini food processor (Black & Decker, Model HC31x) for 30 seconds. A suspension was then prepared in distilled water, with a ratio of 5 g of solid:50 mL of water, kept under magnetic agitation for 10 minutes. The suspension is centrifuged at 3000 rpm/10 minutes, retracting the supernatant.

2.3.1 Photometric quantification of betalains

To determine the dye content present in each material, spectrophotometric readings were performed in the ultraviolet-visible region (□ = 200 – 900 nm) of the aqueous beet extracts.

According to Gonçalves et al. (2015), red beetroot (*Beta vulgares L*) has been used as the main source of betalains (red) and betaxanthins (yellow), and the pigments, betanin (margent) and vulgoxanthin (yellow) are the most abundant, which is why these two dyes were selected as model molecules for the quantification of dyes present in lyophilized beets (JACKMAN and SMITH, 1992; MEGARD, 1993; STRACK, VOGT and SCHLIEMANN, 2003). The betanin and vulgoxanthin contents of the extracts were determined by spectrophotometry using the Nilsson method described by Koubaier (2013). The pigment concentration of the extract samples of 5% (m/v) in relation to the lyophilized material was determined by measuring absorbance at wavelengths of □ = 476 nm and □ = 536 nm, for betanin and vulgoxanthin, respectively (BOVI, 2019). The betalain content (BL) and was calculated as:

$$BL\ (mgL^{-1}) = \frac{A \times DF \times MM \times 1000}{\varepsilon \times L} \qquad (1)$$

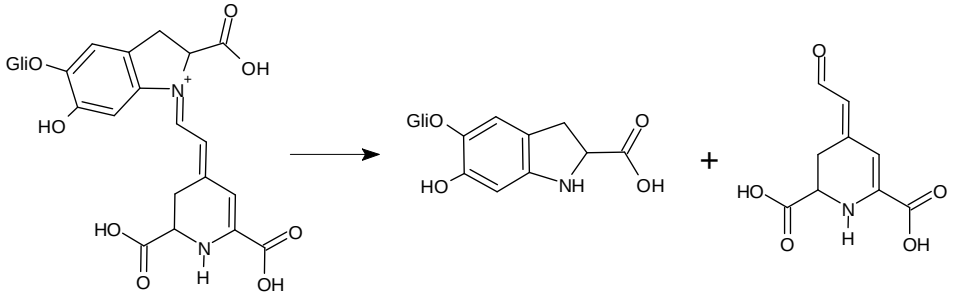
Where: *A* – absorbance of the solution (u.a.); *DF* - dilution factor; *MM* – molar mass of the dye (g $^{mol-1}$); *L* - optical path of the cuvette (1 cm); □ – molar absorptivity (□) (g.mol-1 $^{cm-1}$)

For the quantification of betacyanins (Bc) and betaxanthins (Bx), the molecular molar masses (MM) and molar absorptivity (*ε) were 339 g.mol-1, 48000 L mol -1.cm-1 in H2O: λ =* 475 nm for vulgaxanthin and 550 g.mol-1 and 60000 L.mol-1.cm-1 in H2O: $\lambda = ^{536}$ nm for betanin (GONÇALVES, 2012).

2.4 KINETIC STUDY OF THE DEGRADATION OF BEET DYES

The stability of betalains and betaxanthins is strongly influenced by the presence of light, oxygen, water activity, pH and temperature variation, with cyclodopa-5-O-glucoside and betalamic acid as main products, the latter being a yellow product (SCHIOZER, 2007).

Figure 4. Betanin decomposition reaction.



Source: SCHIOZER, 2007

The thermal degradation of dyes, in general, follows a 1st order kinetics for homogeneous phase reactions (FOGLER, 2010, FROMENT, 2011, ARNAOUT, 2007, SANTOS, 1990) and can be described by the following equation.

$$\ln[C] = \ln[C]_0 - kt \quad (2)$$

Where: [*C*] is the concentration of the dye (mol L-1) and k *is reaction rate constant (*min-1)

Since, according to the Lambert–Beer law, absorbance is a linear function of concentration

$$A = \varepsilon.c.L \quad (3)$$

Where: L – length of the optical path (1 cm); □ - molar absorptivity of betacyanin dye (L mol -1cm-1); c – molar concentration of betacyanin dye (mol L-1), we can assume that the behavior of the concentration of the solution over the time of thermal degradation will be the same as the absorbance as a function of time, so by directly writing the absorption in place of the concentration, we have

$$\ln[A] = \ln[A]_0 - kt \quad (4)$$

which describes the decrease in concentration/absorbance over the thermal degradation reaction time.

This decrease affects the visual presentation of the solution. This, in general, is the first contact of the consumer with the food, receiving special emphasis on color and format (TEIXEIRA, 2009), with direct influence on its acceptability. The color is a parameter still related to the quality of the food, because it refers the consumer to previous experiences with the food, generating expectations as to the appearance that the food should have (KONICA MINOLTA 2021), and the loss of the color of the food is often associated with the loss of quality. The half-life time (*t1/2*) is a parameter that indicates the time in which the initial concentration of the dye is reduced by half, under the reaction conditions, is given by

$$t_{1/2} = \frac{\ln 2}{k} \quad (5)$$

for 1st order reaction.

The effect of temperature on the reaction rate constant can be assessed using the Arrhenius equation.

$$k = k_0 e^{\frac{-\Delta E_a}{RT}} \quad (6)$$

Where: *k0* is the frequency factor of effective shocks for reaction (u. a.) and □*Ea* = the apparent activation energy (J/mol).

The *values of k* determined, for different reaction temperatures, being □$T \geq 20$°C, according to Van't Hoff's rule (FIGUEIREDO, 1987), are used to determine the activation energies for the decomposition reaction of the dyes, by means of the Arrhenius equation (6), as proposed by Vannice (2005).

For kinetic tests, the extract was centrifuged at 3000 rpm/20 minutes and diluted (1:3) to the corresponding ratio of 5×10-3gbeet.mL-1. In a flat-bottomed three-mouthed balloon containing 500 mL of the dye solution, it was placed in a jacketed beaker connected to a thermostatized bath (Solab, model 152/10). The thermal degradation of the extract was monitored by spectrophotometric analysis in the ultraviolet-visible region (Analyser spectrophotometer Model LGS53 UV-VIS Spectrometer, 1 cm quartz bucket). Aliquots of 3 ml of the extract were collected with the aid of a pipette, deposited in a test tube and immersed in an ice bath. The time interval between sample samples was 10 minutes. The test temperatures were 30°C, 60°C and 80°C.

From the absorbance data obtained for the wavelengths (λ) of 475 nm and 536 nm, the kinetic parameters of the degradation of the dyes of the beet extract were obtained.

3 RESULTS AND DISCUSSION

3.1 PHYSICOCHEMICAL CHARACTERIZATION OF BEET

By the characterization of freeze-dried beetroot it is clear that the components of beetroot are concentrated in relation to beetroot *Fresh* (TACO, 2011) without significant losses compared to the contents initially present in the root, a behavior also observed by Bovi *et al* (2019), when using freeze-drying drying.

Table 1 presents the characteristics of the lyophilized beet evaluated.

Table 1. Physicochemical properties of freeze-dried beets.

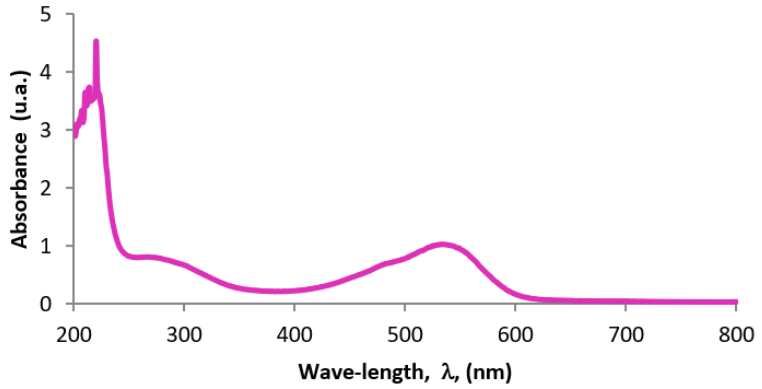
Property	Value*
Humidity (%)	5.24 ± 0.02
Protein (%)	6.29 ± 0.15
Fibres (%)	2.93 ± 0.21
Acidity (mg acetic acid/g)	0.74 ± 0.04

Source: prepared by the author, 2023.

3.2 SPECTROPHOTOMETRIC ANALYSIS OF BEET EXTRACT

Spectrophotometric scanning analysis of the lyophilized beet extract was performed in the range of 200 to 800 nm, as shown in Figure 5.

Figure 5. UV-vis spectrum of beet extract, at pH 6. 20 g/100 mL, dilution factor (1:3).



Source: prepared by the author, 2023.

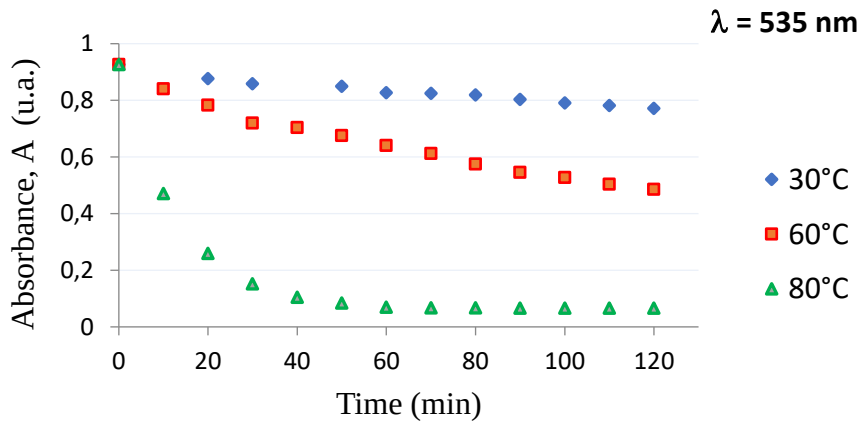
From the spectrophotometric analysis of the extracts obtained with the ratio of 5g of powder:50 mL of H2O, after dilution of the extract (1:3) (dilution factor 4), the concentration of betalain (betanin) found was 35.76 mg $^{L-1}$. The content of betaxanthin (vulgoxanthin) determined was 18.05 mg $^{L-1}$. These concentrations are equivalent to 1.42 mg of betanin and 0.72 mg of vulgoxanthin per gram of lyophilized beet, respectively. As for the betalain content found in beets, this is consistent compared to that found by Vitti *et al*. (2003) of 35mg/100g for fresh beetroot.

The amounts of betaxanthin and vulgoxanthin are in accordance with the work of Schiozer (2007), Gonçalves (2007), Koubaier (2013), Mikołajczyk-Bator (2017) and Azeredo (2009), who attest to these dyes as the most abundant in table beets.

3.3 KINETIC STUDY OF THE THERMAL DEGRADATION OF BEET DYES: BETANIN AND VULGOXANTHINE I

NFigure 5 shows that the decay of the dye concentration in the extract at 30°C is practically linear and less intense than those observed at higher temperatures. This behavior corresponds to that expected for reactions with 1st order kinetics (FOGLER, 2010; Smith, 1990; FROMET, 2011; ARNOUT, 2007).

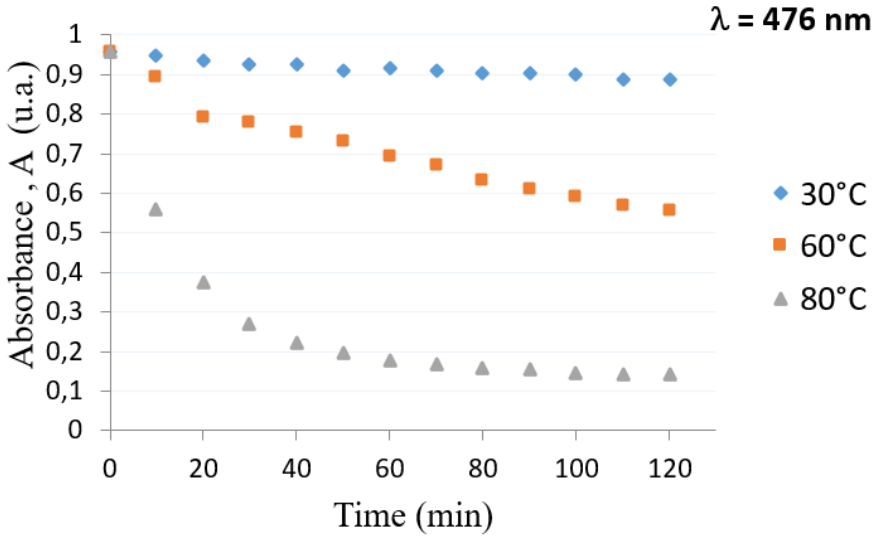
Figure 5. Thermal decomposition of beet betacyanin dye (= 535 nm) in aqueous medium.



Source: prepared by the author, 2023.

Compared to betanin, vulgoxanthin has higher temperature resistance (Figure 6), with less pronounced decay at 30°C, 60° and 80°C. This also exhibits a 1st order reaction behavior in homogeneous phase (FOGLER, 2010).

Figure 6. Thermal decomposition of betaxanthin dye (= 476 nm) of beetroot in aqueous medium.



By the integral method, the kinetic parameters for thermal decomposition of beet dyes were determined. These are listed in Table 2 below.

Table 2 – Reaction rate constant (*k*) and half-life time (*t1/2*) for thermal degradation of vulgoxanthin dye (λ = 476 nm) and betanin (λ = 536 nm) in aqueous medium.

	λ = 476 nm					**λ = 536nm**				
T (°C)	***k*** **(min^{-1})**	***t1/2*** **(h)**	***k0***	**Ea (kJ mol^{-1})**	***R2***	***k*** **(min^{-1})**	***t1/2*** **(h)**	***k0***	**Δ*Ea*** **(kJ mol^{-1})**	***R2***
30	$6,0\times10^{-4}$	19,25	18,23	64,829	0,993	$1,4\times10^{-3}$	8,25	15,48	56,06	0,924
60	$4,3\times10^{-3}$	2,40				$5,1\times10^{-3}$	2,26			
80	$2,4\times10^{-2}$	0,48				$3,7\times10^{-2}$	0,30			

The value of the reaction rate constant 1.4×10^{-3} min^{-1} for betanin it is about 2 times higher than that observed for vulgoxanthin, which is more resistant under reaction conditions at 30°C. The half-life of these two dyes is not more than 1 (one) hour, at 80°C, reinforcing the recommendation that their use in food should be limited to processes at milder temperatures, or with shorter exposure times.

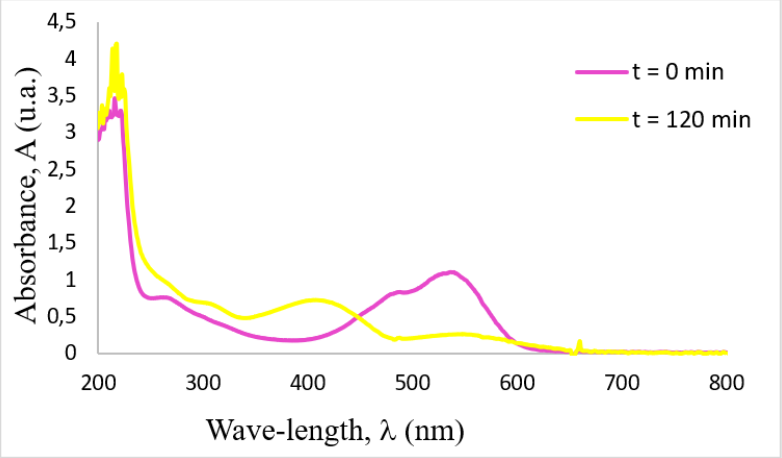
Longaray (2014) reports constant reaction rate, *k, with mean values of 0.027 to 0.0069 min-1 for decomposition of* betaxanthins and from 0.0044 to 0.0067 min-1 for decomposition of betacyanins in water, at a temperature of 75°C to 85°C, with ohmic heating. This difference in the magnitude of reaction rate constants is due to the fact that heating in an aqueous medium, by convective heat transfer, happens more irregularly than ohmic heat.

The activation energy (*ΔE*) calculated for the degradation of the dyes was 56.06 kJ/mol for betacyanins and 64.829 kJ/mol for betaxanthin, which demonstrates that the action of temperature on betanin is more intense than that observed for the dye vulgoxanthin. This can also be seen through the *values of ko*, where the effective shock number 18.23 for vulgoxanthin denotes a smaller number of molecules with minimal energy required to decompose.

The thermal decomposition of beet dyes is accompanied by the regeneration of betalamic acid in the medium, where the solution acquires a yellow color at the end of the process, as seen in Figure 7.

In the UV-vis analysis of the reaction medium after the thermal degradation step, we can see that the spectrum of the initial extract, has the absorption bands at = 476 and 536 nm, which are progressively suppressed (pink spectrum).

Figure 7. Degradation of beet dye in aqueous medium at 80°C.



Source: prepared by the author, 2023.

The appearance of an absorption band at 430 nm corresponds to betalamic acid, yellow in color, resulting from the decomposition of betacyanin. The absorption band 320 nm, corresponds to the glycosylated fragments of dyes, such as cyclo-dopa-5-O-glucoside constituent of betanin (BASTOS, 2012).

4 CONCLUSIONS

The degradation of beet extract dyes is strongly affected by temperature, but presents relative stability at temperatures of 30°C or lower in aqueous medium.

The color of the beet extract presents great stability in the acidic pH range, presenting great potential for applicability in minimally processed foods or with low temperature or low processing time and with temperature conditions storage bands.

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